



RESEARCH PAPER

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## Persistence of protective anti-HBs antibody levels and anamnestic response to HBV booster vaccination: A cross-sectional study among healthcare students 20 years following the universal immunization campaign in Italy

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### ABSTRACT

Vaccination against Hepatitis B Virus (HBV) became mandatory in Italy for all newborns and 12 years-old individuals in the 1991. The immunogenicity of HBV vaccine and the effectiveness of the universal immunization strategy have been widely demonstrated. However the need to assess the antibody concentrations above the well known serological correlate of protection for HBV infection ( $\geq 10$  mIU/mL), established in individuals immunized with a 3 doses vaccination course, is still recommended in subjects exposed to occupational risks in different settings, particularly the healthcare services. This practice has to be performed during the preventive medical examination, before the worker's exposure to biological hazards, as a fundamental part of Occupational Health Surveillance Programs in several Countries, including Italy: the goal is to assure individual protection, also providing booster doses when needed, after many years following the primary vaccination. During the 2011–2013 period, an observational study was performed in Healthcare students (HCSs) trained at a regional university acute-care hospital in North-Western Italy, properly immunized against HBV during infancy or adolescence, in order to evaluate the persistence of seroprotection and to assess the anamnestic response to booster vaccination. Data from 717 subjects undergoing HbsAg Ab and HbC Ab testing during the preventive medical examination, and receiving a booster dose of HBV vaccine when resulting with a non-protective titer ( $< 10$  mIU/mL), were collected and analyzed. Most of the HCSs (74.6%) included in the survey, mean age 24.8 y ( $\pm 4.6$  SD), had received the primary vaccination course during the first year of life (3–5–11 months). Globally, 507 (70.7%) HCSs showed protective antibody titres, and an anamnestic response was observed in more than 95% subjects receiving the booster dose. Our study demonstrated the long-term persistence of protection of HBV vaccine, more than 20 y following the primary immunization, in HCSs who are exposed to occupational health risk. The anamnestic response observed in non-seroprotected subjects who received the booster further confirms the capability of the HBV vaccine to create a strong immunological memory.

### KEYWORDS



anamnestic response;  
Hepatitis B Virus (HBV);  
occupational health  
surveillance programs;  
persistence of protection;  
vaccine

### Introduction

Hepatitis B Virus (HBV) represents a leading cause of acute and chronic liver diseases. To prevent HBV infection and its serious complications, including cirrhosis and hepatic cancer, safe and effective vaccines have been available since the 80s and have largely contributed to the control of the disease in Italy.<sup>1,2</sup> In particular, this latter country was one of the first, in 1983, to implement specific immunization strategies to improve the protection against HBV infection among individuals belonging to risk-categories due to behavioral and professional risks of exposure, including healthcare workers (HCWs).<sup>3</sup> Moreover, a universal vaccination of infants and 12-year-old adolescents became mandatory in 1991.<sup>4</sup> This campaign, with a 3 doses vaccine schedule, reached millions of children in the following

years, with immunization rates approximately of 95%: only when the first cohort of infants immunized in 1991 reached the age of 12 years, universal vaccination was stopped in this target and continued as mandatory among newborns.<sup>5,6</sup>

Although public health policies allowed to control HBV transmission in Italy, some professional categories such as HCWs, but also Healthcare students (HCSs), still need to be considered at high risk for HBV exposure<sup>7–9</sup> with a probability of contracting infection 4 time greater than that reported in the general population.<sup>10</sup> Since 1993, a broad legislative framework, including the Italian Law Decree 81/2008, has been ruling vaccination policy against HBV in HCWs, requiring employers to make available safe and effective vaccines, free of charge, for professionals exposed to biological risk.<sup>11,12</sup>

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In this scenario, the routine detection of anti-HBsAg antibodies titer in serum together with the vaccination against HBV for all susceptible HCWs and HCSs, are currently recommended by the Italian Vaccine Prevention Plan 2012–2014 in order to guarantee protection in these risk categories.<sup>13,14</sup> The optimal immunogenicity elicited by the HBV vaccine has been well demonstrated in several clinical studies in children, adults and adolescents, both when given as a monovalent or combined formulations, even in co-administration with other vaccines recommended by the immunization programs of many Western Countries; also the decline of the HBsAg Ab titers following the primary immunization course over time, possibly at concentrations less than protective, has been reported in the literature, even in long-term follow-up studies, and has been largely investigated in order to understand its clinical meaning.<sup>15–18</sup>

With respect to this relevant issue, despite encouraging results from some studies in the occupational settings have been recently published, particularly among HCWs,<sup>19</sup> few data are available to confirm the long-term persistence of protective anti-HBs antibody levels against HBV infection in HCSs exposed to biological risk after 2 decades from the beginning of the universal immunization campaign against HBV in Italy.<sup>10,20</sup>

In order to investigate this point, we performed an observational study in HCSs followed within the Occupational Health Surveillance Program at IRCCS AOU San Martino – IST teaching hospital in order (i) to measure and compare the persistence of protective anti-HBs antibody levels in HCSs properly immunized during infancy or in adolescence, according to the Italian immunization schedules recommended by the Ministry of Health (3 doses immunization course given at 3–5 and 11 months of life in infants and at time 0 and 1 and 6 months later in adolescents) and (ii) to assess their anamnestic response to HBV booster vaccination when needed, according to current Italian legislation and specific recommendations by the Ministry of Health,<sup>11,13</sup> and (iii) to study the variables associated with a protective anti-HBs antibody level at the first preventive medical examination and associated with a sustained antibody response to the administration of the HBV vaccine booster dose.

## Results

From January 2011 to January 2013, 717 HCSs met the inclusion criteria, properly followed all the procedures foreseen in the study protocol, and gave a written informed consent. The majority of the HCSs were females (481/717, 67.2%) and had a mean age of 24.8 (4.6 SD) years. Most (73.6%) of subjects enrolled were

**Table 1.** Demographic characteristics and anti-Hbs features of Healthcare Students attending the Medical and Pharmaceutical School of the University of Genoa, Italy (Total Vaccinated Cohort).

	n (%)
No of subjects	717 (100)
Age (mean, SD)	24.8 (4.6)
Gender, Female	481 (67.2)
Medical Students	189 (26.4)
Healthcare Professional Students	528 (73.6)
Years since vaccination(mean,SD)	21.35 (3.8)
Vaccinated in infancy	535 (74.6)
Vaccinated in adolescence	182 (25.4)
HBs titer Total Cohort Vaccination $\geq$ 10 mIU/mL	507 (70.7)

**Table 2.** Characteristics of vaccinated Health Care Students attending the Medical and Pharmaceutical School of the University of Genoa, Italy, stratified by age at vaccination.

	Vaccinated in infancy n (%)	Vaccinated in adolescence n (%)	p-value
N° of subjects	535	182	
Age (mean, SD)	23.2 (1.6)	29.7 (6.8)	< 0.001
Gender, Female	367 (68.6)	114 (62.6)	0.14
Years since vaccination	22.8 (1.7)	17.2 (5.2)	< 0.001
Attending medical school	132 (24.7)	57 (31.3)	0.08
HBs titer $\geq$ 10 mIU/mL	358 (66.9)	149 (81.9)	< 0.001

attending healthcare professional Courses (Table 1). The proportion of students resulting seroprotected was 70.7% (507/717) and a lower prevalence of subjects with a non-protective anti-HBsAg antibody titers (< 10 mIU/mL) was found among vaccinees in the adolescent group compared with the infant group ( $p < 0.001$ ) (Table 2). A significant difference also emerged in the comparison between the 2 age-groups, stratifying data according to anti-HBsAg antibody titers. A higher concentration of protective anti-HBs antibody was registered among vaccinees during adolescence ( $p < 0.001$ ) (Table 3): more than 50% of subjects vaccinated in this age-group had titers at values  $\geq$  100 mIU/mL.

All subjects who tested unprotected at the preventive medical examination were required to undergo a HBV vaccine booster dose: 210/717 (29.3%) received it 2 weeks later. An anamnestic response to the booster was reported in 95.2% of those regularly retested for assessing the immune response 4 weeks later: this response was observed among all the subjects belonging to the adolescent group. Less than 5% of those with a titer < 10 mIU/mL after the booster dose (all immunized in infancy) needed to complete a second vaccination course.

At univariate analysis, a protective anti-HBsAg antibody titer ( $\geq$  10 mIU/mL) at baseline resulted significantly associated with these variables: age, years since vaccination and immunization during adolescence. The variable “years since vaccination” was excluded from the final multivariate analysis for collinearity. The multivariate logistic regression model demonstrated that only being vaccinated in adolescence was an independent condition significantly associated with a protective anti-HBsAg antibody titer at baseline (Table 4). No variables resulted significantly associated with sustained antibody response (anti-HBsAg antibody titer  $\geq$  100 mIU/mL) after the administration of the booster HBV vaccine dose.

## Discussion

The present study assessed the immunological performance of HBV vaccination after 2 decades since the beginning of the national immunization program, updating the state of the art on this relevant issue in Italy.

In our survey, a high proportion (70.7%) of the HCSs, enrolled in the study at the preventive medical examination, had persistence of seroprotective levels against HBV after more than 20 y since the administration of primary vaccination course. This finding support the current knowledge on the long-term persistence of anti-HBsAg antibody titers at seroprotective levels in subjects properly immunized against HBV according to the vaccination programs in Italy.<sup>18</sup>

**Table 3.** Characteristics of vaccinated Health Care Students attending the Medical and Pharmaceutical School of the University of Genoa, Italy, stratified by anti-HBs titer.

	HBs titer <10 mIU/mL	HBs titer 10–100 mIU/mL	HBs titer >100 mIU/mL	p-value
No of subjects	210	254	253	
Age (mean, SD)	24.4 (5.3)	24.5 (4.1)	25.6 (4.3)	< 0.001
Gender, Female	140 (66.7)	173 (68.1)	168 (66.4)	0.9
Years since vaccination (mean, SD)	22.1 (2.9)	21.8 (3.2)	20.3 (4.7)	0.02
Attending medical school	49 (23.3)	58 (22.8)	82 (32.4)	0.02
Vaccinated in infancy	177 (84.3)	201 (79.1)	157 (62.1)	< 0.001
Vaccinated in adolescence	33 (15.7)	53 (20.9)	96 (37.9)	

After stratifying the study population in subjects vaccinated in infancy or adolescence, a significant difference in the proportion of seroprotected subjects emerged: a higher prevalence of seroprotected have been registered among those vaccinated in adolescence (81.9% vs 66.9%,  $p < 0.001$ ). This result is lower than those obtained in 2 recent Italian studies that showed a proportion of seroprotected subjects vaccinated in infancy and adolescence of nearly 77% and 88% respectively, after similar period since HBV vaccination.<sup>10,20</sup> However, previous studies have reported that 40–60% of subjects vaccinated in infancy had a seroprotective anti-HBs level after 15–20 y since vaccination, meanwhile the prevalence rise to 85–90% in those vaccinated in adolescence after 10 y since vaccination.<sup>21,22</sup> The immune response in subjects vaccinated during infancy showed a physiological decline in terms of anti-HBsAg antibody concentrations, a well known phenomenon already reported.<sup>18</sup> The antibody concentrations decline observed in our study didn't impact on the protection of vaccinees against HBV, thanks to the immunological memory acquired with the infant immunization course.

Noteworthy, at the multivariate logistic regression model analysis being vaccinated in adolescence resulted the only condition significantly associated with a protective anti-HBsAg antibody titer at baseline.

Furthermore, an optimal capability of the HBV booster vaccine to elicit a strong anamnestic immune response was demonstrated, particularly in subjects receiving the primary immunization course during adolescence.<sup>19</sup> Indeed, in our study, more than 95% of subjects receiving an HBV vaccine booster dosed demonstrated a seroprotective anti-HBs antibody titer after 2 weeks from vaccination, and all subjects vaccinated in adolescence reached a seroprotective anti-HBs antibody titer. The very high (>95%) response to the booster dose is reassuring in the case of professional exposure to wild-type HBV, even in professionals with non-protective HBsAg antibody titers at the time of the accident.<sup>23</sup> However, a recent Cochrane Review stated there is no scientific evidence to reject

or support the need of a booster dose among immunocompetent subjects in the community.<sup>24</sup>

On the other hand, a very small sample of subjects resulted “non-responder” to HBV vaccination after the completion of the second immunization cycle (data not showed).

This survey has some limitations, namely: the intrinsic limits of the study design itself, the poor data obtained in the study population with respect to specific conditions and behaviors (i.e., obesity, alcohol consumption, or smoking habits) that are considered variables involved in the decrease of the immunogenicity of HBV vaccine over time,<sup>25</sup> and the fact that not all the HCSs were compliant with the procedures of the study protocol and were consequently discharged.

Our findings strongly support the recommendation by the regulatory health Agencies to screen and booster susceptible individuals belonging to high-risk groups, such as professionals employed in health care services. Some recent studies performed in this occupational setting in our country, among both HCWs and HCSs trained at teaching hospitals, reported similar encouraging evidences.<sup>10,20</sup>

Moreover serological screening, of all HCSs, and administration of booster HBV vaccine doses when needed, are preventive policies particularly meaningful in the current period when foreign students coming from countries with potential gaps in the immunization programs or coverage rates could increase the reservoir of young susceptible workers exposed to biological risks.

In conclusion, well planned vaccination strategies implemented during the last decades, together with ongoing Healthcare Surveillance Programs, largely contributed to the control of HBV infection and its associated complications in Italy, both in the community and in several occupational settings at high-risk, particularly the healthcare one, optimizing the primary prevention of this serious biological agent.

Finally, the present study demonstrate the good persistence of protection against HBV in a large sample of HCSs exposed to occupational health risk during the training programs, more than 20 y following the primary immunization courses. The

**Table 4.** Variables independently associated with seroprotection against HBV among Healthcare Students attending the Medical and Pharmaceutical School of the University of Genoa, Italy.

Variable	Univariate Analysis			Multivariate Analysis	
	< 10 IU/L (n = 210) n (%)	≥ 10 IU/L (n = 507) n (%)	P-value	OR (95% CI) ≥ 10 IU/L vs < 10 IU/L	P-value
Age (mean, SD)	24.4 (5.3)	25.1 (4.3)	0.09	0.98 (0.94 – 1.03)	0.4
Gender, Female	140 (66.7)	341 (67.3)	0.88		
Years since vaccination (mean, SD)	22.1 (2.9)	21 (4.1)	0.09		
Attending medical school	49 (23.3)	140 (27.6)	0.24		
Vaccinated in adolescence	33 (15.7)	149 (29.4)	< 0.001	2.57 (1.53 – 4.46)	< 0.001

optimal anamnestic response to HBV booster vaccination observed in non-seroprotected subjects further confirms the capability of the vaccine to create a strong immunological memory. All these encouraging findings support current practices adopted within the Healthcare Surveillance Programs for HCWs and undergraduate healthcare students in several Western countries, confirming their fundamental role for the control of HBV transmission in the healthcare setting.

## Materials and methods

### Study design

The study was observational, cross-sectional, and used routine demographic, clinical, and laboratory data collected within the Occupational Health Surveillance Program.

### Setting and study population

The study was performed, during the 2011–2013 period, in HCSs attending the Medical and Pharmaceutical School of the University of Genoa and trained at the IRCCS AOU San Martino-IST Teaching Hospital of Genoa, Italy, the regional tertiary adult acute care reference hospital with nearly 1,300 beds. According to the Occupational Health Protocol, all students attending the 3rd year of the Medicine and Surgery Course (medical students) and the first year of Nursing, Pediatric Nursing and Midwifery Courses (healthcare professional students) were actively summoned before the exposure to biohazard in different Departments of the hospital. All the students included in the study met the following inclusion criteria at enrollment: born in the Genoa metropolitan area; HBsAg/anti-HBc negative and born from HBsAg-negative mothers; vaccinated according the Italian universal HBV immunization campaign when infants or adolescents with a full 3 doses immunization course; having documentation of vaccination, in the form of a certificate or booklet generated by Local Health Unit of Genoa. Exclusion criteria included serological evidence of HBV infection (i.e., positivity for anti-HBc antigen or other HBV-related antigens), presence of chronic diseases and congenital or acquired immune disorders.

### Procedures and definition

All participants were actively recruited within the Occupational Health Surveillance Program of the IRCCS AOU San Martino-IST Teaching Hospital, before potential exposure to biological risks, during the preventive medical examination, according to the Occupational Health Protocol. Detailed information about health status and previous HBV vaccination course, with proper certification, were obtained, and a blood sample for detection of anti-HBsAg antibodies was collected according to current International Standards: subjects with anti-HBsAg concentrations of  $\geq 10$  mIU/ml following the primary series of vaccination are considered protected against HBV infection.<sup>26</sup>

The students with anti-HBs concentrations  $\geq 10$  mIU/mL were classified as protected against HBV, while those with values  $< 10$  mIU/mL were required to undergo a HBV vaccine booster dose: students receiving the booster dose were re-tested

4 weeks later to assess protective antibody response. Individuals continuing to have anti-HBs concentrations  $< 10$  mIU/mL post-booster were offered 2 additional vaccine doses (1 and 6 months after the booster dose), and were re-tested. Only subjects with titer  $< 10$  mIU/mL after 2 complete vaccination courses (6 doses) were considered “non-responders” to HBV vaccine.

HBsAg, anti-HBc and anti-HBs were detected using commercially available immunoassay kits: MonolisaUltra (Biorad Laboratories, Marnes-la-Coquette, France) and EIAGEN (Adaltis Italia, Milano, Italy).

### Ethics

The study was approved by the Ethics Committee of the Liguria Region. All the activities were performed in compliance with the Declaration of Helsinki and current healthcare standards according to the recommendations of the Italian Ministry of Health. All students included in the survey had signed a written informed consent according to routinely healthcare procedures of the Occupational Health Surveillance Program at the IRCCS AOU San Martino-IST Teaching Hospital. Personal information regarding all the subjects included in the investigation was protected according to the Italian law.<sup>27</sup>

### Statistical analysis

All data collected were entered and analyzed using EpiInfo 7.0 (Centers for Disease Control and Prevention, CDC, Atlanta, GA, USA). Additional analyses were performed using the SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Continuous numeric variables were summarized as mean and standard deviation and/or, when appropriate, median and interquartile range. Nominal and ordinal categorical variables were summarized in the form of percentage proportions and 95% confidence interval (CI 95%). Association between continuous numeric variables and the main outcome of interest, were tested using T-student test or non-parametric test, when appropriate. Association between categorical variables and the main outcome of interest, were tested using the Chi-squared test or Fisher exact test, when appropriate.

Variables potentially associated with protective anti-HBsAg antibody titers ( $\geq 10$  mIU/mL) at baseline and variables potentially associated with sustained HBV antibody response (Ab titer  $\geq 100$  mIU/mL) after the administration of the booster dose of HBV vaccine were evaluated with univariate analysis and multivariate logistic model.

Multicollinearity between variables was assessed. All tests were 2-tailed and a P-value  $< 0.05$  was determined to represent statistical significance.

### Abbreviations

HBV	hepatitis B Virus
HCWs	Healthcare workers
HCSs	Healthcare students
NVPP	national vaccination prevention plan (piano nazionale prevenzione vaccinale)

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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